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Original Research

Inhibitory activities of genistein on signaling pathways in pulmonary inflammation induced by cisplatin in experimental rats

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Abstract

Aim: This work was conducted to investigate the inhibitory effects of genistein on various signaling pathways such as nuclear factor kappa-B (NF-κB), prostaglandins (PGs), proinflammatory cytokines and reactive oxygen species (ROS) in pulmonary inflammation induced by cisplatin in rats.

Material and Methods: Sixty adult male albino rats (Sprague-Dawley) were separated into five groups: G1, control; G2, received single interperitoneal dose of CP (2.5mg/kg) to induce pulmonary inflammation (PI); G3, G4, and G5, rats received three levels of genistein (10, 50, 150 mg/kg/day respectively) orally via CP injection.

Results: Treatment with GEN improved the lung tissue levels of oxidative stress biomarkers (SOD, CAT, GSH, MDA), inflammatory cytokines (IFN-γ, IL-6, TNF-α), fibrogenic and apoptotic markers as compared to CP group. Rats received genistein at high dose (150 mg/kg/day) had the most significant improvement in serum levels of IL-1β, NF-kB, IL-10, CRP, PGE2 followed by the medium dose (50 mg/kg/day).

Discussion: The study results demonstrated the ameliorative effect of GEN against pulmonary inflammation induced by CP. Genistein could inhibit the oxidative stress, reduce the accumulation of free radicals, and suppress the production of inflammatory cytokines.

Keywords

Genistein, Cisplatin, Signaling Pathways, Pulmonary Inflammation, Cytokines, Oxidative Stress

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Introduction

Inflammation is a defense mechanism of the immune system towards infection or injury. Inflammation serves to remove toxic and foreign stimuli and to rebuild tissue integrity and physiological function. Severe lung failures such as acute lung damage, respiratory distress syndrome, and alveolar edema may result from inflammatory pulmonary illness. Increased endothelial cell permeability, alveolar injury, pulmonary edema, and the aggregation of inflammatory cells into lung tissue are the hallmarks of acute pulmonary inflammation. Tissue injuries will also worsen in highly inflammatory conditions [1]. Recent research has revealed that alveolar and bronchial epithelial cells are essential for the pathological development of acute respiratory distress syndrome and pulmonary fibrosis, and that the recovery from lung damage may depend on the normal, timely regeneration of epithelium structure and function [2].

Convincing anticancer drug cisplatin (CP) is frequently used to treat a variety of malignancies, such as testicular, ovarian, bladder, lung, and kidney cancers. It has detrimental toxic side effects despite having a strong anti-tumoral activity. Oxidative stress, which impacts the lungs and other tissues and organs, is one of the negative effects that have been described. Significant side effects from cisplatin chemotherapy have also been recognized, including interstitial inflammation, fibrosis, and structural lung damage [3]. These unfavorable consequences of cisplatin-induced lung damage could result from cisplatin's capacity to initiate oxidant-induced fibrotic and inflammatory lesions in the lungs. [4]. In addition, cisplatin stimulates the production of certain inflammatory chemokines and cytokines, including as TNF-α and TNF-α which are thought to participate in the progression of the inflammatory process,

Anti-inflammatory remedies that are currently used are nonsteroidal anti-inflammatory drugs. However, these medications often have a number of negative effects when used over an extended period of time [5]. Therefore, it's critical to develop a novel anti-inflammatory medication that treats inflammation more effectively, safely, and at a reasonable cost. Genistein (GEN) is well known for its anti-inflammatory, anti-tumor, and antioxidant properties. It may also shield the human body against lung damage [6]. Genistein is a member of the isoflavones subgroup within the flavonoid family. It's a phytoestrogen mostly found in legumes. GEN is a chemical component found in nature with a similar structural similarity to mammalian estrogens. It is believed that genistein has numerous positive health effects, including anticancer properties, protection against osteoporosis, and a lower risk of cardiovascular disease [7]. GEN has a protective effect against breast cancer and shown to inhibit NF-κB expression in the nucleus of breast cancer cell lines [8]. In addition, genistein clearly reduces inflammation by influencing lymphocytes, monocytes, and granulocytes; this provides a new possibility for the development of phytotherapeutic drugs that could be used in anti-inflammatory treatments. [9].

Material and Methods

Chemicals

Cisplatin: 1mg/ml sterile concentrate colorless to pale yellow solution, purchased from Hospira Company (UK). All the other chemicals and reagents were analytical grade.

Genistein was purchased as a dietary supplement from Vital Nutrients Co. (Middletown CT, USA). Genistein was first dissolved in dimethyl sulfoxide (DMSO) then diluted with physiological saline (0.9% sodium chloride). Each rat from the genistein treated groups received no more than 0.2% DMSO, corresponding to 10 µl. In the control and PI-treated groups, each rat received physiological saline with 10 µl DMSO.

Experimental Animals and design

Adult male albino rats weighing 102±9 g were used in this study. The rats were acclimatized to laboratory conditions for three days prior to initiation of experiments, then divided and housed in environmentally controlled cages (24±1 0C, 45±5% humidity and 12 h light/dark cycle). They were fed commercially available diet, and tap water was given ad libitum. Sixty male rats were divided into five groups (12 rats/group) as follows:

Group 1: Rats fed a standard diet and received only 1ml saline/ day (Control).

Group 2: Pneumonitis group (CP): Rats fed standard a balanced diet and received a single intraperitoneal dose of CP (2.5mg/kg) to induce pulmonary inflammation.

Group 3: Low genistein group (CP+ GEN 1); Rats fed a balanced diet and received genistein (10 mg/kg b.w/day) by oral gavage after 3 days of CP injection.

Group 4: Medium genistein group (CP+ GEN 2); Rats fed a balanced diet and received genistein (50 mg/kg b.w/day) by oral gavage after 3 days of CP injection.

Group 5: High genistein group (CP+ GEN 3); Rats fed a balanced diet and received genistein (150 mg/kg b.w/day) by oral gavage after 3 days of CP injection.

At the end of the experimental period (8 weeks), rats were fasted overnight, then anesthetized with ether, blood samples were obtained from the hepatic portal vein then transferred into centrifuge tubes. To obtain serum for the biochemical analysis, tubes were centrifuged at 10000 x g for 10 minutes at 25°C. Serum samples were collected and stored in dry clean plastic tubes at -20°C until used for various biochemical analyses. The lung of each animal was removed by dissection, washed by ice-isotonic saline, and blotted between two filter papers. Part of the lungs were homogenized in 9 volumes of 0.1 M potassium phosphate-buffered saline at pH 7.4. Homogenates were centrifuged at 5000 rpm for 15 minutes; aliquots of supernatant were kept at −20 °C for the biochemical determinations.

Biochemical Analysis

1-Lung Tissue Analysis

Pro-Inflammatory cytokines

Lung tissues tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), and interleukin -6 (IL-6) were determined by enzyme-linked immunosorbent assay following the instruction of the manufacturer using ELISA kits (Kamiya Biomedical Co. CA. USA).

Oxidative stress markers

Reduced glutathione (GSH), superoxide dismutase (SOD), catalase activity (CAT), and malondialdehyde (MDA) in lung tissues were evaluated spectrophotometrically using (Biovision Kit, CA. USA).

Fibrogenic and apoptotic markers

The concentration of fibrinogen (FGN) and P53 in lung tissues

was estimated by Enzyme Linked Immunosorbent Assay (ELISA) sandwich technique using kit purchased from (Bioassay Technology Laboratory, Shanghai, China).

2-Serum Analysis

Inflammatory cytokines

Serum nuclear factor kappa-B (NF-κB), interleukin-1β (IL-1β), and interleukin-10 (IL-10) were determined by enzyme-linked immunosorbent assay following the instruction of the manufacturer using ELISA kits (Kamiya Biomedical Co. CA. USA).

Pro-Inflammatory mediators

Serum levels of C-reactive protein (CRP), prostaglandin E2 (PGE2), and nitric oxide (NO) were determined by kits purchased from Biodiagnostic Co. Ltd, Cairo, Egypt.

Statistical Analysis

Data were statistically analyzed by SPSS version 20.0 statistical packages. Data were represented as the means ± SE; statistical differences between groups were performed using t-test. Differences were considered significant at $p \leq 0.01$.

Ethical Approval

This study was approved by the Ethics Committee of Sciences Academy of Experimental Research, Egypt [15 May 2023, No. 44305].

Results

Lung tissue levels of IFN-γ, IL-6, and TNF-α were elevated significantly ($p<0.01$) in rats administrated with CP compared with the control group and gradually normalized by receiving genistein (Table 1). Results in Table 2 denoted that CP induced oxidative stress represented as a significant ($p \leq 0.01$) decrease in lung tissue SOD, CAT and GSH activities and elevation of MDA level as compared to control group. On the other hand, treatment with genistein improved the levels of oxidative stress biomarkers. In the same context, results showed that rats received genistein at high dose (150 mg/kg/day) had the most significant improvement in oxidative stress biomarker levels followed by the rats received genistein at medium dose (50 mg/kg/day). Cisplatin increased levels of pro-inflammatory mediator markers as compared to the control group. Meanwhile, genistein treatment significantly downregulated nitric oxide, and CRP levels depending on the given doses (by 14.1%, 18.9%, and 33.3%, for NO and 12.6%, 56.1%, and 66.7% for CRP respectively), as compared with the CP group. Furthermore, Genistein treatment significantly decreased PGE2 level (by 18.3%, 36.5%, and 45.8%, respectively) compared with CP group (Table 3). Cisplatin has a significant effect on serum levels of pro-inflammatory cytokines (IL-1β, NF-kB, IL-10). The increased levels of cytokines were detected following interperitoneal dose of CP as compared to the control group. Additionally, (Figure 1) showed that, the values of serum IL-1β, NF-kB, and IL-10 cytokines were significantly lower in rats receiving moderate and high doses of GEN than those observed in rats given a lower dose of GEN. Results revealed the activation in fibrogenic and apoptotic markers in CP treated rats as observed by significant increase in FGN and P53 levels as compared to the control group. Treatment with genistein at a high dose modulated the production of both fibrogenic and apoptotic markers as compared to low and moderate doses of genistein (Figure 2).

Discussion

Inflammation is a biological reaction in the immune system that can be brought on by pathogens, toxic compounds, and other factors [10]. In recent years, studies have increasingly found that alveolar epithelial cells play an active role in inducing fibroblast proliferation and activation, which could lead to the destruction of lung structure and pulmonary inflammation [11]. Therefore, a key factor in the effective regression of lung inflammation may be the ability to promptly restore the structure of the alveolar epithelial barrier. In the present study, administration of CP led to a significant elevation in lipid peroxidation level in lung tissue that was revealed by increasing

Table 1. Effect of all treatments on lung tissue levels of pro-inflammatory cytokines

Groups	IFN-γ (ng/ g tissue)	IL-6 (ng/ g tissue)	TNF-α (ng/ g tissue)
Control (C)	32.65 ^{a} ± 3.8	117.6 ^{a} ± 8.3	149.3 ^{a} ±7.1
Pulmonary inflammation (CP)	78.35 ^{b} ±6.5	255.3 ^{b} ± 9.1	295.3 ^{b} ±11.0
Low Genistein (CP+ GEN 1)	62.22 ^{c} ±4.1	245.5 ^{c} ± 11.2	215.3 ^{c} ±9.5
Med Genistein (CP+ GEN 2)	35.30 ^{a} ±2.8	132.8 ^{d} ± 6.8	170.5 ^{d} ±5.7
High Genistein (CP+ GEN 3)	37.33 ^{d} ±5.8	127.2 ^{d} ± 7.3	189.5 ^{d} ±8.3

Data are expressed as mean+/- SE, (n= 12). Significance was made using One Way ANOVA test (LSD). Means that bearing different letters (a, b, c, d, e) are significantly at ($p \leq 0.01$)

Table 3. Effect of all treatments on serum levels of inflammatory mediators

Groups	Nitric Oxide (µmol/L)	PGE2 (pg/mL)	CRP (mg/L)
Control (C)	51.5 ^{a} ±2.5	218.3 ^{a} ± 11.4	3.99 ^{a} ±0.5
Pulmonary inflammation (CP)	83.3 ^{b} ±5.8	437.6 ^{b} ± 25.6	13.2 ^{b} ±1.2
Low Genistein (CP+ GEN 1)	71.5 ^{c} ±3.1	357.3 ^{c} ± 16.3	12.4 ^{b} ±1.1
Med Genistein (CP+ GEN 2)	67.5 ^{c} ±4.3	277.8 ^{d} ± 10.9	6.22 ^{c} ±0.8
High Genistein (CP+ GEN 3)	55.5 ^{a} ±2.8	227.1 ^{a} ± 13.5	4.72 ^{a} ±0.9

Data are expressed as mean+/- SE, (n= 12). Significance was made using One Way ANOVA test (LSD). Means that bearing different letters (a, b, c, d, e) are significantly at ($p \leq 0.01$)

Table 2. Effect of all treatments on lung tissue levels of oxidative stress biomarkers

Groups	MDA (nmol/g tissue)	SOD (U/ g tissue)	GSH (mg/ g tissue)	CAT (U/ g tissue)
Control (C)	28.2 ^{a} ±1.5	330.0 ^{a} ±16.0	59.6 ^{a} ±7.1	199.0 ^{a} ±11.0
Pulmonary inflammation (CP)	63.9 ^{b} ±6. 5	145.1 ^{b} ±8.2	29.0 ^{b} ±4.6	73.2 ^{b} ±5. 5
Low Genistein (CP+ GEN I)	52.1 ^{c} ±3.4	184.5 ^{c} ±11.6	39.5 ^{c} ±3.5	103.5 ^{c} ±6. 3
Med Genistein (CP+ GEN 2)	41.1 ^{d} ±1.1	219.1 ^{d} ±12.8	42.3 ^{c} ±6.5	179.3 ^{d} ±7.1
High Genistein (CP+ GEN 3)	31.8 ^{a} ±5.6	290.1 ^{e} ±15.1	46.5 ^{d} ±5.3	181.2 ^{d} ±9.6

Data are expressed as mean+/- SE, (n= 12). Significance was made using One Way ANOVA test (LSD). Means that bearing different letters (a, b, c, d, e) are significantly at ($p \leq 0.01$)

MDA level. Additionally, resulted in a significant decline in antioxidants detected by decreased levels of GSH, and SOD as compared to control group. Cisplatin causes the production of ROS and oxygen free radicals, including hydroxyl radicals, superoxide anions, and hydrogen peroxide. A hydrogen atom from the polyunsaturated fatty acids in membrane lipids is taken up by the hydroxyl radical, which starts lipid peroxidation. When these radicals interact with macromolecules like proteins, nucleic acids, and membrane lipids, they can cause significant tissue damage.

On the other hand, GEN therapy suppressed MDA level and augmented GSH and SOD activities. Studies have previously recognized the anti-oxidative properties of isoflavones such as genistein [12]. Genistein has been shown to protect cells from over-production of reactive oxygen species (ROS) by scavenging free radicals. This prevents NF-κB activation, which is crucial for the development of cytokine and inflammation. [13]. Consequently, genistein is a good choice for PI treatment due to its capacity to trigger cell signaling pathways that may prevent ROS production. The current study also revealed that CP administration resulted in vigorous inflammatory responses as evidenced by marked increment in lung tissue TNF-α, IFN-γ, IL-6 and serum levels of IL-1β, NF-kB, and IL-10 as compared to control group. Cisplatin stimulates the expression of several inflammatory chemokines and cytokines, including TNF-α, and causes significant cellular damage. Cytokines such as TNF-α may function as triggers to activate NF-κB, and p65 is crucial for NF-κB activation [14]. Activation of NF-κB directly induces the inflammatory response by triggering the transcription of various genes in different innate immune cells, including chemokines, cytokines, and adhesion molecules. In addition, NF-κB involved in the inflammation indirectly by triggering the regulation of cell proliferation, apoptosis, morphogenesis, and differentiation as well as promoting the development of inflammatory T cells [16]. The NF-κB represents a family of inducible transcription factors that is essential for several immunological and inflammatory response mechanisms [15]. Based on these findings, one potential therapeutic strategy for the treatment of inflammatory diseases and tissue damage could be to effectively regulate the NF-κB signaling pathway. Therefore, a compound with an inhibitory effect on NF-κB activation may be the potential applicant of a new anti-

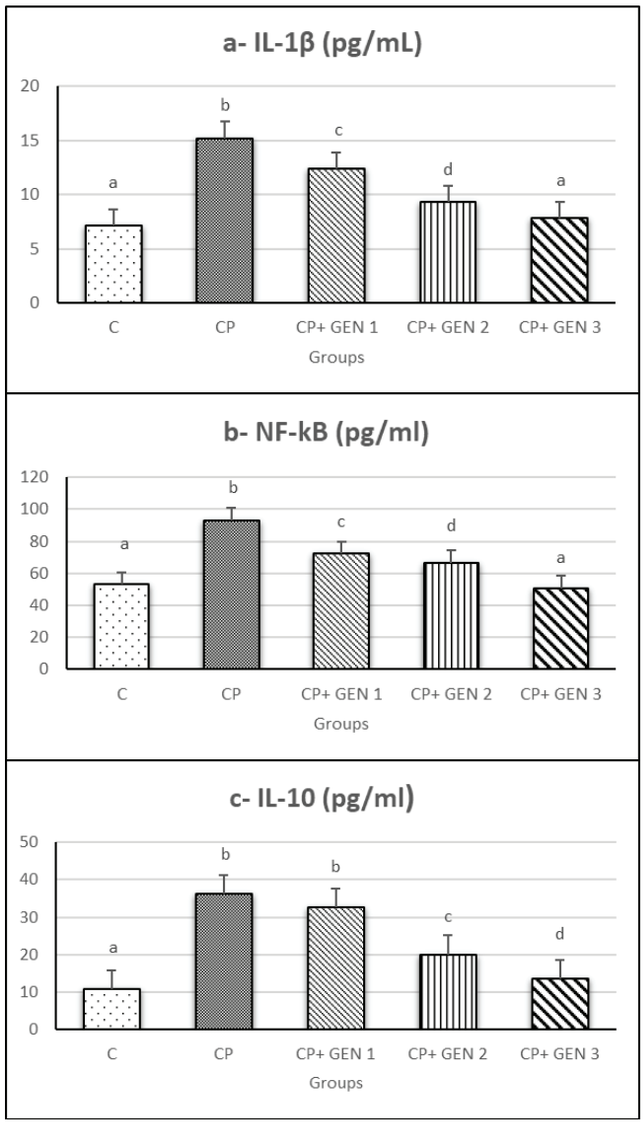

Figure 1. Effect of all treatments on serum levels of pro-inflammatory cytokines (IL-1β , NF-kB, IL-10) respectively. The P values were calculated using the One-Way ANOVA test. The abbreviations mean; C: control, CP: cisplatin, GEN 1: low dose of genistein, GEN 2: a medium dose of genistein, GEN 3: high dose of genistein. Means that bearing different letters (a, b, c, d) are significantly at (p≤0.01)

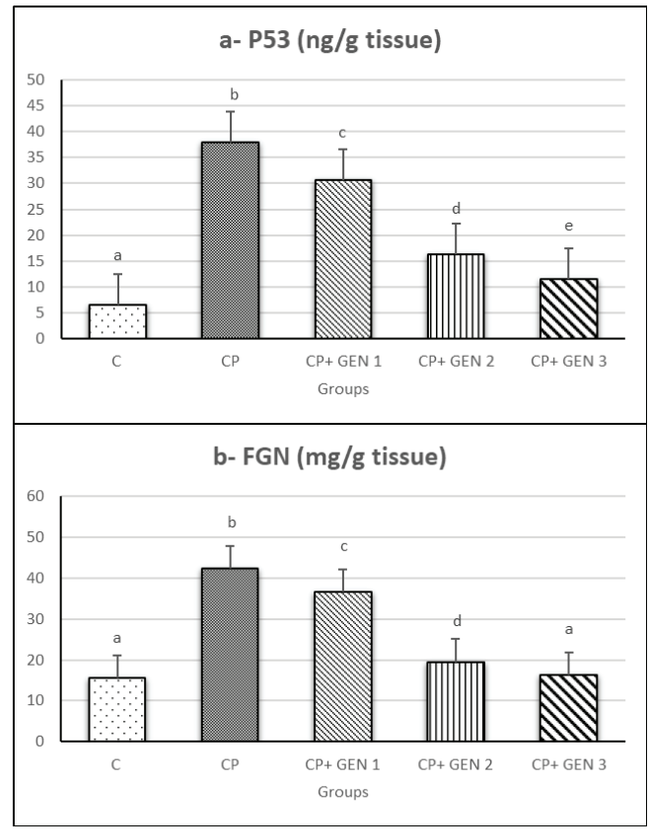

Figure 2. Effect of different treatments on lung tissue apoptotic and fibrinogenic biomarkers (P53 and FGN) respectively. The P values were calculated using the One-Way ANOVA test. The abbreviations mean; CL control, CP: cisplatin, GEN 1: low dose of genistein, GEN 2: medium dose of genistein, GEN 3: high dose of genistein. Means that bearing different letters (a, b, c, d, e) are significantly at (p≤0.01)

inflammatory agent.

Genistein has been shown to have a suppressive effect on the production of proinflammatory cytokines. These results may help identify possible courses of action for treating chronic inflammatory disorders [17]. Pharmaceutical inhibition of TNF-α and IL-6 is an excellent example of how to apply an approach to suppress inflammatory cytokines. According to earlier studies, genistein is a highly pleiotropic molecule that can interact with various cellular targets involved in conditions of hyper-inflammation. Genistein was also demonstrated to have protective effects on lung structure and to drastically lower inflammatory and apoptotic markers in ovariectomized diabetic rats [18]. Numerous mechanisms have been shown to be involved in the action of genistein to reduce inflammation. One such mechanism is the downregulation of NF-κB, which results in a decrease in the expression of TNF-α, IL-1, and IL-6. [19].

In response to inflammation and cell damage, the serum level of C-reactive protein (CRP) rapidly and significantly increases. CRP could be involved in the regulation of lung function and might contribute to the pathogenesis of various pulmonary complaints. TNF-α and IL-6 levels were raised in CP-induced rats in response to these pro-inflammatory cytokines, which in turn raised CRP levels [20]. Results of the present study showed that a significant suppression in the levels of the inflammatory mediators TNF-α, IFN-γ, IL-6 and CRP was observed following the administration of CP rats with GEN. It was demonstrated that in cell culture supernatants from peripheral blood mononuclear cells, genistein dramatically inhibited IFN-γ production [21]

The other factors which have a central role in inflammation are prostaglandin E2 (PGE2) and nitric oxide (NO). It has been shown that genistein could inhibit lipopolysaccharide induced PGE2 production in over-activated macrophages [22]. PGE2 is involved in the generation of the inflammatory response which their biosynthesis is significantly increased in inflamed tissue. PGE2 is abundant throughout the body and exhibits versatile biological functions. It plays an important role in inflammation as it is involved in all processes leading to the classic signs of inflammation, including redness, swelling, and pain. Results of the current study demonstrated that genistein treatment significantly downregulated NO, and CRP levels depending on the given doses and decreased PGE2 levels as compared to CP rats. Therefore, PGE2, which contributes to the generation of the inflammatory response, may be the candidate drug target for anti-inflammatory therapy. Similarly, a recent study examined how genistein affects the prostaglandins pathway through inhibition of PGE2 release, decreasing PG receptor expression, and inhibiting COX-2 expression [23]. The current results indicated an activation in fibrogenic and apoptotic markers in CP treated rats as observed by significant upregulation in FGN and P53 levels as compared to control group. The main cellular target of CP is to deform the natural structure of the DNA which cause DNA damage in cells, block cells division and result in apoptotic cell death [24]. The cytotoxic activity of CP is attributed to numerous mechanisms including inflammation, apoptosis, and necrosis, which affects the lungs and various other tissues and organs. Apoptosis is a form of cell death, and as such it has been frequently induced by chemotherapy agents Cisplatin-induced damages are considered to be an important trigger of p53 activation that leads to cell apoptosis. Increased cisplatin-induced p53 activation, resulting in apoptotic cell death. [25]. TNF –α and IL-6 appear to play a central role in the development of the acute phase processes during inflammation and share the ability to induce acute-phase proteins such as fibrinogen.

Results of the present study showed that genistein treatment at a high dose modulated the production of both fibrogenic and apoptotic markers as compared to CP rats. The reduction in the inflammatory mediators caused by treatment with GEN could in turn cause the inhibition of fibrinogen. Since genistein has been indicated to downregulate cytokine-induced signal transduction pathways in the immune system cells, we hypnotized that it may also exert anti-inflammatory effects on pneumonitis induced by cisplatin.

Conclusion

In conclusion, the study results demonstrated the ameliorative effect of GEN against pneumonitis induced by CP. GEN could inhibit oxidative stress, reduce the accumulation of free radicals, and suppress the production of inflammatory cytokines. This study mainly emphasized the anti-inflammatory activity of genistein to provide direction in the discovery of potential novel, safe and efficacious natural anti-inflammatory agents in the future.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and Human Rights Statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or compareable ethical standards.

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Conflict of Interest

The authors declare that there is no conflict of interest.

References

1. Ngamsri KC, Müller A, Bösmüller H, Gamper-Tsigaras J, Reutershan J, Konrad FM. The pivotal role of CXCR7 in stabilization of the pulmonary epithelial barrier in acute pulmonary inflammation. The Journal of Immunology. 2017;198(6):2403-13.

2. McClendon J, Jansing NL, Redente EF, Gandjeva A, Ito Y, Colgan SP, et al. Hypoxia-inducible factor 1α signaling promotes repair of the alveolar epithelium after acute lung injury. The American Journal of Pathology. 2017;187(8):1772-86.

3. Leo F, Pelosi G, Sonzogni A, Chilosi M, Bonomo G, Spaggiari L. Structural lung damage after chemotherapy: Fact or fiction?. Lung Cancer. 2010;67(3):306-10.

4. Geyikoglu F, Isikgoz H, Onalan H, Colak S, Cerig S, Bakir M, et al. Impact of high-dose oleuropein on cisplatin-induced oxidative stress, genotoxicity and pathological changes in rat stomach and lung. Journal of Asian Natural Products Research. 2017;19(12):1214-31.

5. Pok LS, Shabaruddin FH, Dahlui M, Sargunan MS, Said AR, Lau S, et al. Risk of upper gastrointestinal adverse events in Malaysian rheumatic patients on long-term non-steroidal anti-inflammatory drugs. Int. J. Clin. Rheumatol. 2018;13(3):172-78.

6. Liu FC, Wang CC, Lu JW, Lee CH, Chen SC, Ho YJ, et al. Chondroprotective effects of genistein against osteoarthritis induced joint inflammation. Nutrients. 2019;11(5):1180-93.

7. Chen X, Wu Y, Gu J, Liang P, Shen M, Xi J, et al. Anti-invasive effect and pharmacological mechanism of genistein against colorectal cancer. Biofactors. 2020;46(4):620-8.

8. Mukund V, Behera SK, Alam A, Nagaraju GP. Molecular docking analysis of nuclear factor-κB and genistein interaction in the context of breast cancer. Bioinformation. 2019;15(1):11-17.

9. Goh YX, Jalil J, Lam KW, Husain K, Premakumar CM. Genistein: A review on its anti-inflammatory properties. Frontiers in Pharmacology. 2022;13(1):820969-81.

10. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, et al. Inflammatory responses

and inflammation-associated diseases in organs. Oncotarget. 2018;9(6):7204-18.
11. Maldonado M, Salgado-Aguayo A, Herrera I, Cabrera S, Ortíz-Quintero B, Staab-Weijnitz CA, et al. Upregulation and nuclear location of MMP28 in alveolar epithelium of idiopathic pulmonary fibrosis. American Journal of Respiratory Cell and Molecular Biology. 2018;59(1):77-86.
12. Mazumder MA, Hongsprabhas P. Genistein as antioxidant and antibrowning agents in in vivo and in vitro: A review. Biomedicine & Pharmacotherapy. 2016;82(1):379-92.
13. Russo M, Russo GL, Daglia M, Kasi PD, Ravi S, Nabavi SF, et al. Understanding genistein in cancer: The "good" and the "bad" effects: A review. Food chemistry. 2016;196(1):589-600.
14. Huang Y, Chen J, Jiang T, Zhou Z, Lv B, Yin G, et al. Gallic acid inhibits the release of ADAMTS4 in nucleus pulposus cells by inhibiting p65 phosphorylation and acetylation of the NF-κB signaling pathway. Oncotarget. 2017;8(29):47665-74.
15. Liu T, Zhang L, Joo D, Sun SC. NF-κB signaling in inflammation. Signal transduction and targeted therapy. 2017;2(1):1-9.
16. Shi J, Yang G, You Q, Sun S, Chen R, Lin Z, et al. Updates on the chemistry, processing characteristics, and utilization of tea flavonoids in last two decades (2001-2021). Critical Reviews in Food Science and Nutrition. 2023;63(20):4757-84.
17. Tahmasebi S, El-Esawi MA, Mahmoud ZH, Timoshin A, Valizadeh H, Roshangar L, et al. Immunomodulatory effects of nanocurcumin on Th17 cell responses in mild and severe COVID-19 patients. Journal of Cellular Physiology. 2021;236(7):5325-38.
18. Daghigh F, Alihemmati A, Karimi P, Habibi P, Ahmadiasl N. Genistein preserves the lungs of ovariectomized diabetic rats: Addition to apoptotic and inflammatory markers in the lung. Iranian Journal of Basic Medical Sciences. 2017;20(12):1312-17.
19. Sutrisno S, Wulandari RC, Sulistyowati DW, Wulandari RF, Wahyuni ES, Yueniwati Y, et al. Effect of genistein on proinflammatory cytokines and estrogen receptor–β in mice model of endometriosis. Asian Pacific Journal of Reproduction. 2015;4(2):96-9.
20. Sato K, Takigawa N, Kubo T, Katayama H, Kishino D, Okada T, et al. Effect of a Cyclooxygenase-2 Inhibitor in Combination with (−)-Epigallocatechin Gallate or Polyphenon E on Cisplatin-Induced Lung Tumorigenesis in A/J Mice. Acta Medica Okayama. 2023;77(1):65-70.
21. Mace TA, Ware MB, King SA, Loftus S, Farren MR, McMichael E, et al. Soy isoflavones and their metabolites modulate cytokine-induced natural killer cell function. Scientific reports. 2019;9(1):5068-78.
22. Hämäläinen M, Nieminen R, Asmawi MZ, Vuorela P, Vapaatalo H, Moilanen E. Effects of flavonoids on prostaglandin E2 production and on COX-2 and mPGES-1 expressions in activated macrophages. Planta Medica. 2011;77(13):1504-11.
23. Hegab II, Abd-Ellatif RN, Sadek MT. The gastroprotective effect of N-acetylcysteine and genistein in indomethacin-induced gastric injury in rats. Canadian Journal of Physiology and Pharmacology. 2018;96(11):1161-70.
24. Mohamed NA. Myelosuppression and nephrotoxicity induced by cisplatin in female rats: The role of berberine nanoparticles. Journal of Advances in Biology. 2018;11(1):2218-35.
25. Tchounwou PB, Dasari S, Noubissi FK, Ray P, Kumar S. Advances in our understanding of the molecular mechanisms of action of cisplatin in cancer therapy. Journal of Experimental Pharmacology. 2021:13(1):303-28.

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